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Pharmacognostic Studies of the Bark of *Kandelia candel* (L) Druce

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Abstract: *Kandelia candel* Druce (Rhizophoraceae) is one of the true mangrove species found in South- East Asian mangrove communities, and it is reported that the bark of the plant along with pepper or dried ginger and rose water is found beneficial for the treatment of diabetes. Our aim and objective of the present work was to study pharmacognostic and physio-chemical standards for the bark of the plant *Kandelia candel*. It includes the macroscopy, microscopy, and powder microscopic characteristics of the bark which provide valuable information for the identification of bark. The barks show greyish to reddish-brown colour, it is slightly curved, and sometimes recurved inner the surface shows slightly yellowish red. The histological section of the bark shows the epidermal layer and mostly it is broken and shows periderm, periderm consists of suberise dead phellem cells, several layers of circular cells found in the cortical region, the interior of the bark shows wide radially oblong aerenchyma tissues, calcium oxalate crystals are very common in the bark which is found as druces and prismatic type crystal forms. Powdered drugs showed the presence of fragments of periderm, cortical parenchyma cells, calcium oxalate crystals, and fibres. Physiochemical constants like ash values, extractive values, foaming, swelling indexes, and fluorescent analysis were performed on the powdered drug which is fairly constant for crude drugs it helps to ascertain the purity of the drug. Preliminary phytochemical screening was performed and it reveals the presence of steroids, phenols, tannins, terpenoids, and flavonoids which may be responsible for the pharmacological effect of the bark of *Kandelia candel*, the results of the study help in setting the standards for the bark of *Kandelia candel* which can be useful for the identification and authentication of drugs in future.

Keywords: *Kandelia candel, Microscopy, Physico Chemical Constants, Mangroves and Pharmacognosy*

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1. INTRODUCTION

Medicinal, as well as aromatic plants are necessary resources of all-natural medications which are generally expanded in the woodlands as well as cultivated; they have progressed along with human civilization. From the moment numerous herbs were made use for the therapy of human disorders as well as conditions due to the fact that they include the therapeutically crucial components. Raising the intricacy of different illnesses, the demand for brand-new medicines is of utmost relevance, botanicals and various other natural resources are the potential sources of novel phytoconstituents which have possible to heal one of the most conditions as well as illnesses of human[1]. Mangroves typically thrive under difficult environmental conditions such as severe heat salinity as well as radiation that might not be beneficial for the typical plants to grow. Thus, mangroves have established unique adjustments to continue to be making it through these conditions[2]. Mangroves have a crucial duty in safeguarding the nature it serves as a filter between the sea as well as river and also consequently provide fresh water to the seaside areas, it functions as an eco-friendly wall surface as well as safeguard the sea turbulence by standing up to the winds as well as from various other all-natural catastrophes. Mangrove ecotone nourishes as well as offers food and also a sanctuary for aquatic microorganisms as well as additionally for various other earthbound pets. Mangroves are generally used as firewood and charcoal and their uses also include the construction of dwellings, furniture, boats, and fishing gear, and the production of tannins for dying and leather production. Numerous mangroves are an abundant resource of tannins and also have been made use of for the removal of tannins as well as natural leather production[3].To endure damaging ecological problems there is a feasible modification in their physical procedures which causes the synthesis of brand-new chemical substances; these substances help them to safeguard from numerous diseased conditions of humans. A lot of these substances have a substantial biological activity which can provide the requirements for the unique medicines for the therapy of complicated illnesses in human[4]. Mangroves are abundant resources of secondary metabolites such as alkaloids, phenols, flavonoids, tannins, saponins, glycosides, terpenoids etc. The medicinal values of these metabolites present in mangroves are still not totally made use of in contemporary medicine. Mangroves also provides food and a wide variety of traditional products. It is known that mangroves contain amino acids, vitamins, and minerals which help the growth and development of marine organisms.[5] Several mangroves and mangrove-associated plants were identified and are found to be useful for the treatment of many diseases. The leaves, roots flowers *Rhizophora mucronata* was reported to be useful for the treatment of constipation, elephantiasis, haematoma, hepatitis, febrifuge, gastric motility disorders, diabetes, inflammation, wounds, ulcers, fertility-related and menstruation disorders.[6] *Sonneratia alba* fruits are used for expelling intestinal parasites and help to cure cough, skin injuries, swellings and sprains. *Sonneratia caseolaris* fruits were found to be beneficial for the treatment of sprain, bleeding, haemorrhage, piles and toxicity against mosquito larvae and it also possess astringent and antiseptic properties similarly most of the mangroves had novel phytochemicals they are found to beneficial for the treatment various ailments in humans and animals.[7,8]*Kandelia candel* (L) Druce is the only true mangrove species of the genus Kandelia W. & A. of Mangrove Rhizophoraceae, distributed in South- East Asian mangrove communities. Aerial roots are not usually found in this species but the viviparous germinated seedling is slender up to 45 cm long and tapering sharply towards the end. It grows on the slopes of the river and ridge forests.[9,10] the bark is useful for tanning leather and dyeing. Bark and leaves are used in the treatment of diabetes and also show antioxidant activity.[11,12] The wood is used for temporary constructions. The present study was mainly carried out to identify the pharmacognostic characteristics of the bark of *Kandelia candel* includes macroscopical, microscopical and powder microscopical features and also aimed to establish the physicochemical characters of the bark such as ash values, extractive values, loss on drying, fluorescence analysis and determination of trace elements etc. This will help the identification and authentication of the bark and also for the identification of adulteration with biosimilar materials

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The plant material for the proposed study was procured from Valapattanam, Kannur district in Kerala, India. Plants were collected carefully; only a healthy plant with normal organs were selected for the study and was identified by Dr.C. Murugan, Scientist'& Head of Office, Botanical Survey of India, Southern Regional Centre Coimbatore. Voucher No: BSI/SRC/5/23/2019/Tech/3547.The few pieces of the barks were preserved for the microscopical and powder analysis remaining barks were dried in shade and pulverised for further studies

2.2 Pharmacognostic Studies

Morphological and histological studies were conducted using fresh bark, and coarsely powdered material was used for the analysis of the powder. FAA (formalin-5ml+Acetic acid-5ml+ 70% Ethyl alcohol-90%) is used for fixing the fresh bark. Twenty-four hours after fixing tertiary – butyl alcohol is used for the dehydration of the sample. Infiltration of the specimens was carried by gradual addition of paraffin wax until TBA (tertiary butyl alcohol) solution obtained supersaturation. The paraffin blocks were used for casting the specimen.[13,14] The paraffin-embedded specimens were sectioned with the help of a Rotary Microtome. The sections were dewaxed and the thickness of the section was 10-12µm.[15,16] The sections were stained with Toluidine blue. The dye develops pink colour to the cellulose walls, blue to the lignified cells, dark green to suberin, and violet to the mucilage.[17]. For studying the stomatal morphology, venation pattern, and trichome distribution, paradermal sections, as well as clearing of leaf, were carried out with 5% sodium hydroxide.[18] Glycerine-mounted temporary preparations were made for cleared materials. Powdered materials of bark were cleared with sodium hydroxide and mounted in glycerine medium after staining and different cell components were studied and measured. Photographs of different magnifications were taken with Nikon lab photo 2 microscopic units. For normal observation a bright field was used. For the study of crystals, starch grains, and lignified cells polarized light was employed.[19,20] since these structures have the birefringent property that under polarized light they appear bright against the dark background

2.2.1 Measurement of Fibre Length and Breadth

Materials and reagents: - Compound microscope, eyepiece micrometer, stage micrometer, chloral hydrate solution, phloroglucinol, hydrochloric acid, dilute glycerine
Method: - Calibrated the eyepiece micrometer using the stage micrometer and calculated the factor. Boil a small quantity of powdered drug with chloral hydrate solution. Remove cleared powder in a watch glass and stain with one drop each of phloroglucinol and concentrated Hydrochloric acid. Mounted a little of the treated powder in dilute glycerine and observed the slide under low power.[21]

2.3 Physicochemical andPhytochemical Analysis

Physicochemical studies consist of percentage extractive values, ash values, swelling and foaming index and loss on drying which were determined by standard methods and procedures[22] and Preliminary Phytochemical screenings were conducted after extraction of coarsely powdered bark by successive solvent extraction procedure. The extraction was performed by using solvents Petroleum ether, ethyl acetate and ethanol as per the increasing order of their polarity. After completion of extraction, each extracts were filtered by what man filter paper and the extracts were concentrated by using rotary vacuum evaporator at low pressure and temperature and the presence of phytochemicals were detected by standard methods.[23,24]

2.4 Fluorescence Analysis

Coarsely powdered bark material were mixed with different chemical reagents and exposed to visible, ultraviolet light (Short UV and long UV) to study their fluorescence behaviour.[25,26]

2.5 HPTLC Analysis

Silica gel 60F F254 HPTLC plate 6.0 × 1o cm manufactured by E. MERCK KgaA was used for the analysis. CAMAG Automatic TLC Sampler 4 (ATS4) was used for the application of the sample on plate which was equipped with a 25 µl syringe and 3 µl of the sample solution was applied as bands. After application of the sample, it was placed on CAMAG ADC 20x10cm developing chamber with mobile phase Toluene:ethylacetate:methanol(6:4:1). CAMAG TLC Scanner 3 was used for the densitometric scanning and the CAMAG visualizer was used for the visualization of different bands and the images were taken under visible and UV light of 254nm and 366nm.[27]

3. RESULTS

3.1 Macroscopical Studies

Kandeliacandel bears simple leaves, which are opposite, decussate, 8.5- 12 x3-4.5 cm, stipulate, clustered at shoot apex; lamina oblong or lanceolate, shiny green above and pale green beneath. Flowers white, to 2.2 x3.3 cm in axillary dichotomously branched 4- flowered cymes; calyx white, tub copular, enclosing the ovary; lobes 5, persistent; petals 5, free, white, deeply bilobed, lobes equal with 3-6 cilia at apex; stamens many, free, inserted on the rim of calyx cup, filaments unequal. Fruit a drupe, 1.5-2 cm long, ovoid-conical; peduncle elongating; seed one; hypocotyl to 40 x1.5 cm, spindle-shaped, slightly curved with pointed radical, surface smooth, green; cotyledonary collar protruded and exposed on maturity. The bark appears to be greyish to reddish-brown bark in colour, smooth, and has lenticels. The bark is a slightly curved and sometimes recurved inner surface of the bark slightly yellowish red

3.2 Organoleptic Properties

The bark appears to be greyish to reddish-brown bark in colour, smooth and has lenticels. Bark is slightly curved and sometimes recurved inner surface of the bark slightly yellowish red in colour

Fig 1.1 *Kandelia candel* bark outer surface

Fig 1.2 *Kandelia candel* bark inner surface

3.3 Microscopical Studies

3.3.1 Bark Microscopy

The bark has more or less smooth surfaces, the epidermal layer is broken and the periderm (Pe) is exposed (Fig.3.1, 2). The periderm is 100µm thick. The periderm consisting of 2-5 layers suberised dead phellem (PM) cells which stain brown due to suberin in the cell wall. Inner to the phellem to the phelloderm (Phd) layer which is two or three cells in thickness. The phelloderm cells have cellulose walls and they are living cells. The cortical zone follows the periderm and is very thick comprising several layers of circular cells which are less compact having narrow intercellular spaces (Fig.4.1,2) The inner boundary of cortical zone is marked by a thick cylinder or discrete circular masses of brachysclereids (SC) (Fig.3.2,4.2) further interior of the bark occur wide, radially oblong aerenchyma (Aec) tissue which is characterised by horizontally elongated wide air passages separated by uniseriate reticulate partition filaments (PF) (Fig5.1,2). The cells of the partition layers have dense cell inclusions. On either side of the aerenchymatous passage occur intact and compact secondary phloem (SPh) elements and ground parenchyma cells (GPa). The sieve elements (SE) are small darkly stained and possess small companion cells. Phloem parenchyma cells are fairly large and no prominent cell inclusions are seen in the parenchyma cells. The sieve elements are the outermost, first formed secondary phloem get crushed and collapsed. The collapsed sieve elements appear as dark irregular or horizontal streaks (Fig 6.1). The parenchyma cells in the collapsed phloem (CPh) region are highly dilated, exerting pressure over the sieve elements. The sieve elements in the recently formed secondary phloem are intact and remain uncollapsed. The sieve elements are small, angular and thick walled. Each sieve element has a small companion cell located at the corner of the sieve element (Fig.6.2). Crystal distribution; Calcium oxalate crystals are very common in the bark. Spherical bodies of crystals called druses (Dr) with sharp spines on the surface (Fig. 7.1). The crystals are solitary and are seen in each parenchyma cell of the cortex (Co). The druses appear bright white under polarised light. A second type of calcium oxalate crystals called prismatic (Pcr) type are seen within the cell lumen of the brachysclereids. These crystals are prominent and polyhedral in shape. Only one crystal occurs in a cell (Fig.7.2)

3.3.2 Powder Microscopic Studies

1. The powder preparation of the bark shows small fragments of periderm having phellem and phelloderm derivatives (Fig.8.1). The phelloderm cells are rectangular and thick walled. the phelloderm cells are vertically oblong and thin walled
2. Cortical parenchyma cells (CoP) (Fig.8.2): the cortical tissue consists of circular, compact, darkly stained parenchyma cells (Fig.8.3). The cells are polyhedral and six or more sided. Some of the cells possess calcium oxalate dresses. The crystal is wide and occupies the entire length of the cell.
3. There are also thick masses of cells which include parenchyma cells in addition to sieve elements. These elements are small angular cells with thick walls. There is a small companion cell located at the corner of the sieve element.
4. Libriform fibres are common in the powder. The fibers are of two types found in the powder.

a) Narrow fibre (NFi) (Fig.9.1). long narrow fibers are common in powder. These fibers have narrow cell lumen and thick walls; the fibers are gradually tapering at the ends. The fibers are 1mm long and 10µm thick

b) In addition to the narrow fibers there are also wide fibers (WFi). The wide fibers are short with wider cell lumen. The cell walls are less thick. The wide fibers are 360µm long and 10µm wide (Fig.9.2)

3.3.3 Measurement of Fibre Length and Breadth

Measured the length and width of the stained fiber by focusing them on the lines of the eyepiece micrometer. Note the number of divisions covered by length and width of the fiber. Calculate the values of 15 fibers and multiply them by the factor. Calculated the average value and indicated the range for the width and length of the fibers (Table 1)

Table1. Length and Breadth of the fibre

Sl.No	Fibre length	Fibre breadth	Calibration factor	Fibre length (µm)	Fibre breadth (µm)
	No.of eyepiece division	No.of eyepiece division			
1	56	2	14.02	785.12 ± 0.22	28.04 ± 0.07
2	45	1	14.02	630.9 ± 0.38	14.02 ± 0.19
3	64	1	14.02	897.28 ± 0.45	14.02 ± 0.16
4	42	2	14.02	588.84 ± 0.39	28.04 ± 0.38
5	45	1	14.02	630.9 ± 0.21	14.02 ±0.04
6	25	2	14.02	350.5 ± 0.06	28.04 ± 0.24
7	34	1	14.02	476.68 ± 0. 21	14.02 ± 0.08
8	27	1	14.02	378.54 ± 0.17	14.02 ± 0.12
9	65	2	14.02	911.3 ± 0.35	28.04 ± 0.11
10	32	1	14.02	448.64 ± 0.28	14.02 ± 0.09
11	52	2	14.02	729.04 ± 0.08	28.04 ± 0.13
12	23	1	14.02	322.46 ± 0.12	14.02 ± 0.15
13	36	1	14.02	504.72 ± 0.32	14.02 ± 0.23
14	37	1	14.02	518.74 ± 0.23	14.02 ± 0.31
15	56	2	14.02	785.12 ± 0.05	28.04 ± 0.09
Fibre length- 350.5 ± 0.06-597.25 ± 0.23 -911.3 ± 0.35 µm Fibre breadth - 14.02 ± 0.09-19.63 ± 0.16-28.04 ± 0.09 µm					

Fibre length and breadth were measured highest length and breadth of fibre were measured at 911.3 ± 0.35µm and 28.04 ± 0.09 µm respectively and the lowest length and breadth of the fibre were 350.5 ± 0.06 µm and 14.02 ± 0.09 µm respectively. It is one of the important parameter for the standardisation and authentication of the powdered bark of Kandelia candel

3.4 Preliminary Phytochemical Screening

Coarsely powdered bark material were extracted by successive solvent extraction procedure using petroleum ether, ethyl acetate and ethanol using soxhlet apparatus and performed the preliminary Phytochemical screening using standard reagents and it reveals the presence Tannins, flavonoids, steroids, phenols, terpenoids and glycosides

Table 2 Preliminary phytochemical investigation of the different extracts of the bark of *Kandeliacandel*

	Petroleum ether	Ethyl acetate	Ethanol
Alkaloids	-	-	-
Glycosides	-	+	+
Steroids	+	+	+
Phenolics	-	+	+
Tannins	-	-	+
Flavonoids	-	+	+
Triterpenes	+	+	-
Carbohydrates	-	-	-

+, Present; -, absent

Preliminary phytochemical Screening of the different extracts reveal the presence of steroids in all the extracts, triterpenes were found both in petroleum ether and ethyl acetate fraction of the extracts, glycosides, phenolics and flavonoids were detected in both ethyl acetate and ethanolic extracts and ethanol extract also showed the presence of tannins. The anti-diabetic activity of the *Kandelia candel* bark may be due to the presence of compounds like flavonoids, phenolics, tannins and triterpenes

3.5 Physicochemical Parameters

Physicochemical analysis of the coarsely powdered bark viz. loss on drying, swelling and foaming index, ash values and extractive values were performed as per the standard procedure(Table3)

Table3. Physico *chemical parameters*

Sl.No	Physico chemical parameters	Mean value (% w/w, n=3) ± SD
1	Loss on drying (% w/w)	2.54 ± 0.05
2	Total ash (%w/w)	6.38 ± 0.38
3	Water soluble ash (% w/w)	3.04 ± 0.36
4	Acid insoluble ash (% w/w)	1.02 ± 0.42
5	Water soluble extractive (% w/w)	7.07 ± 0.52
6	Alcohol soluble extractive (% w/w)	9.01 ± 0.86
7	Ether soluble extractive (% w/w)	4.92 ± 0.15
8	Swelling index	2.26 ± 0.44
9	Foaming index	Less than 100

SD-Standard Deviation

The physico-chemical parameters help to authenticate the powdered bark of *Kandelia candel*. It was also found useful for finding out any adulteration with morphologically similar materials, since an increase of the moisture content may enhance the microbial attack and cause the spoilage of drug, ash value indicates the mineral content of drugs, low acid insoluble ash indicate the low contamination with earthy material, extractive values give an approximate estimation of the nature of chemical constituent of the drug, swelling and foaming index shows the amount of mucilage and saponins present in the bark respectively

3.6 The Fluorescence Analysis

Fluorescence analysis of the pulverised *kandelia candel* bark were performed by mixing with various reagents and observed under day light and UV (254nm and 365nm) light and observed their fluorescent behaviour. (Table 4)

Table.4 *Fluorescence analysis*

Sl.No		Daylight	UV (254 nm)	UV (365nm)
1	Powder drug as such	Yellowish brown	Yellowish green	black
2	Powder+Methanol	Yellowish brown	Yellowish red	brown
3	Powder+1% Glacial acetic acid	Yellowish red	Greenish black	Black
4	Powder+10% NaOH	Reddish brown	Dark brown	Black
5	Powder+dil.NH_3	Reddish yellow	black	Black
6	Powder+Conc.HNO_3	Red	Yellowish brown	**Black**
7	Powder+dil.NH_3+Conc.HNO_3	Yellowish red	brownish	black

8	Powder+1M H_2SO_4	Reddish yellow	Greenish yellow	Black
9	Powder+1M Hcl	Yellowish brown	dark brown	black
10	Powder+10% $FeCl_3$	Yellowish brown	Greenish	black
11	Powder+Acetone+Methanol	Yellowish green	Greenish	black
12	Powder+10% Iodine	Yellowish brown	Greenish	black

Powdered bark of *Kandelia candel* after treatment with various reagents shows characteristic colours in visible and UV radiations this may be due to the nature of the chemical constituents present in the drug, it is one of the major criteria for the pharmacognostic standardisation of the powdered drug.

3.7 *HPTLC Analysis*

Visible **366 nm** **254 nm**

Fig 2.1 HPTLC chromatograms visualized under a visible light, UV 254 nm, and UV 366 nm

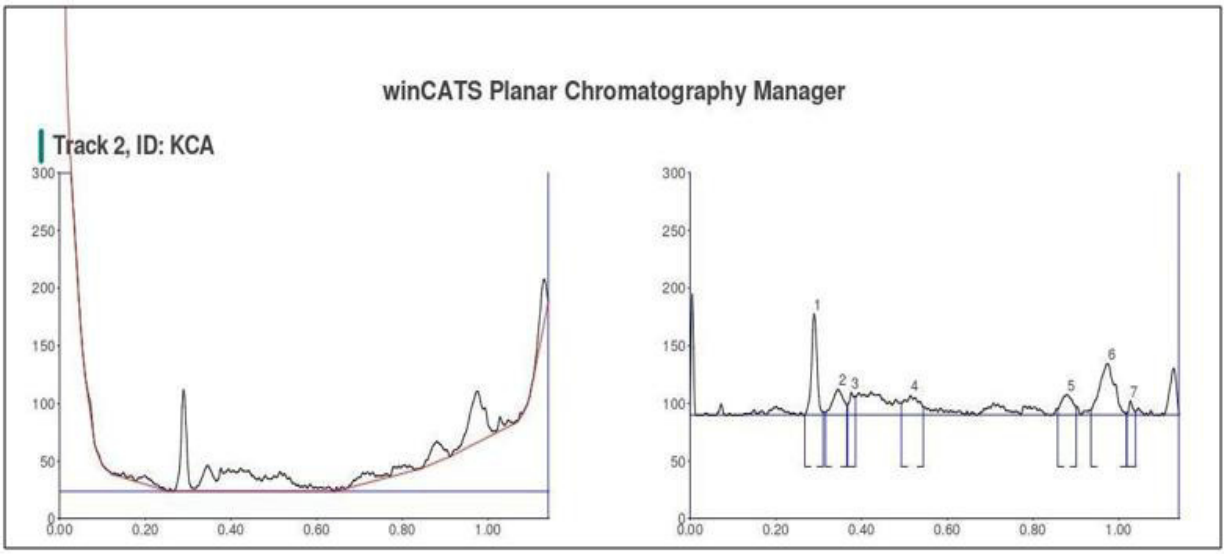

Fig 2.2 HPTLC chromatograms

Chromatogram representing 6 peaks which indicate the presence of different phytoconstituents, each peak on the chromatogram represents a phytoconstituent

Table 5 HPTLC peak table of ethanolic extract of the bark of *Kandeliacandel*

Peak	Start Rf	Start Height	Max Rf	Max Height	Max %	End Rf	End Height	Area	Area %
1	0.27	0.3	0.29	87.9	39.69	0.31	2.7	919.5	24.29
2	0.32	2.1	0.35	22.3	10.05	0.37	8.8	460.0	12.15
3	0.37	9.3	0.38	19.8	8.94	0.39	16.3	209.8	5.54
4	0.49	10.5	0.52	16.9	7.62	0.55	6.9	463.8	12.25
5	0.86	5.0	0.88	17.7	7.99	0.90	7.1	385.3	10.18
6	0.94	4.4	0.98	44.5	20.10	1.02	1.8	1256.0	33.18

The chromatogram was scanned at Visible and UV 254 nm and 366nm representing 6 peaks which indicate the presence of different phytoconstituents, each peak on the chromatogram represents a phytoconstituent. The Rf value calculated was helpful for the identification of different phytochemicals present in the sample by comparing with standards

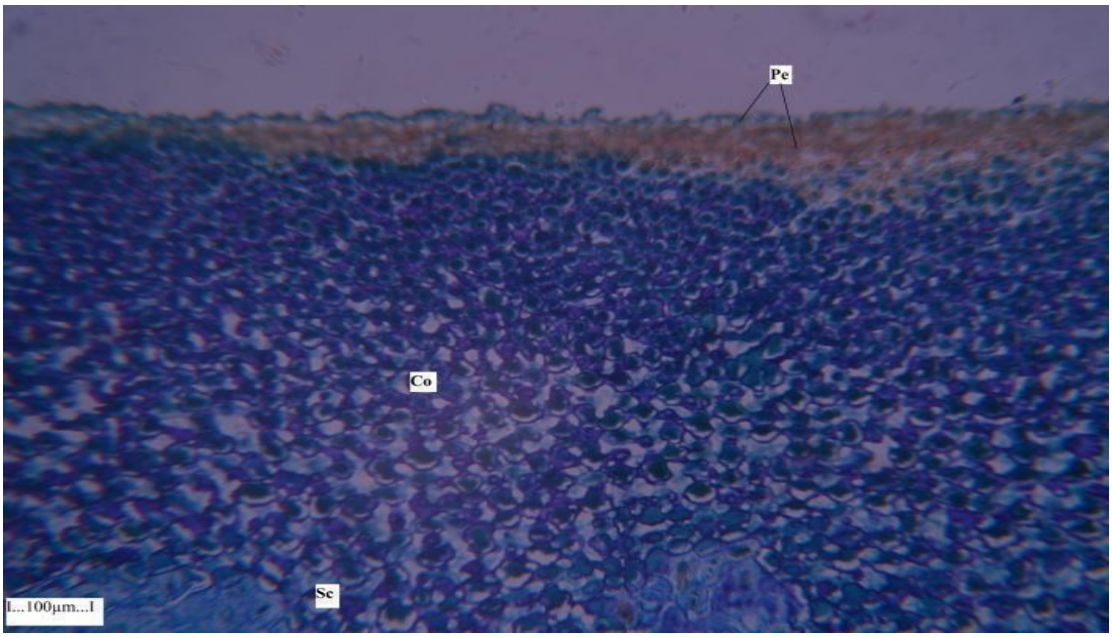

Fig 3.1T. S of outer part of the bark showing periderm and outer cortex

Pe - Periderm, Co - Cortex, Sc - Sclereids

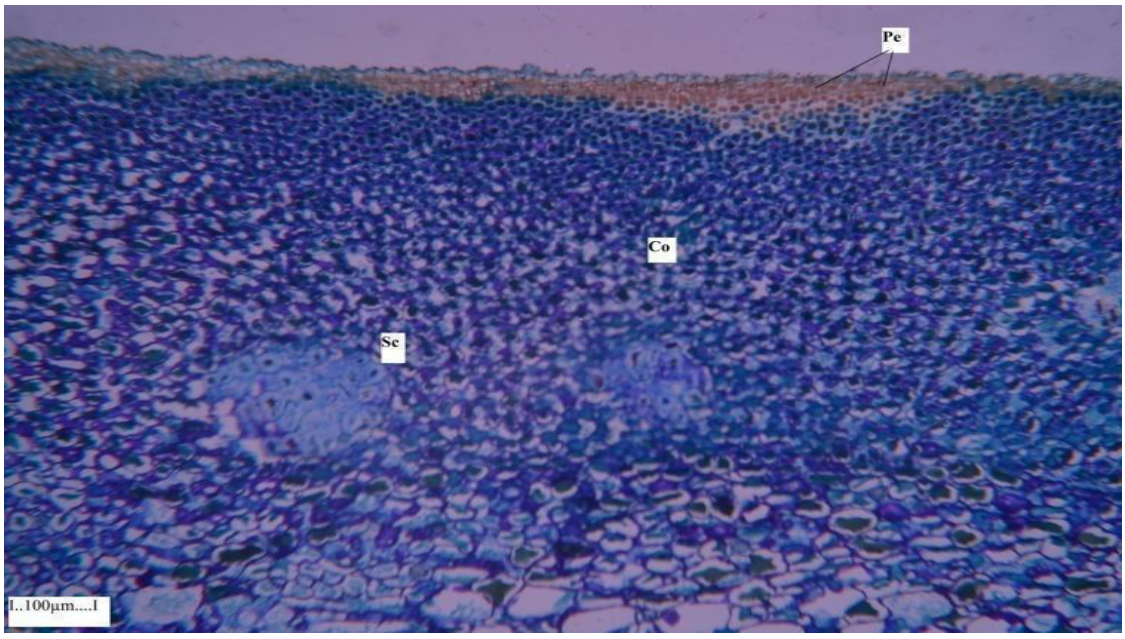

Fig 3.2 T. S of bark showing inner cortex showing circular masses of discrete sclereids

Pe - Periderm, Co - Cortex, Sc - Sclereids

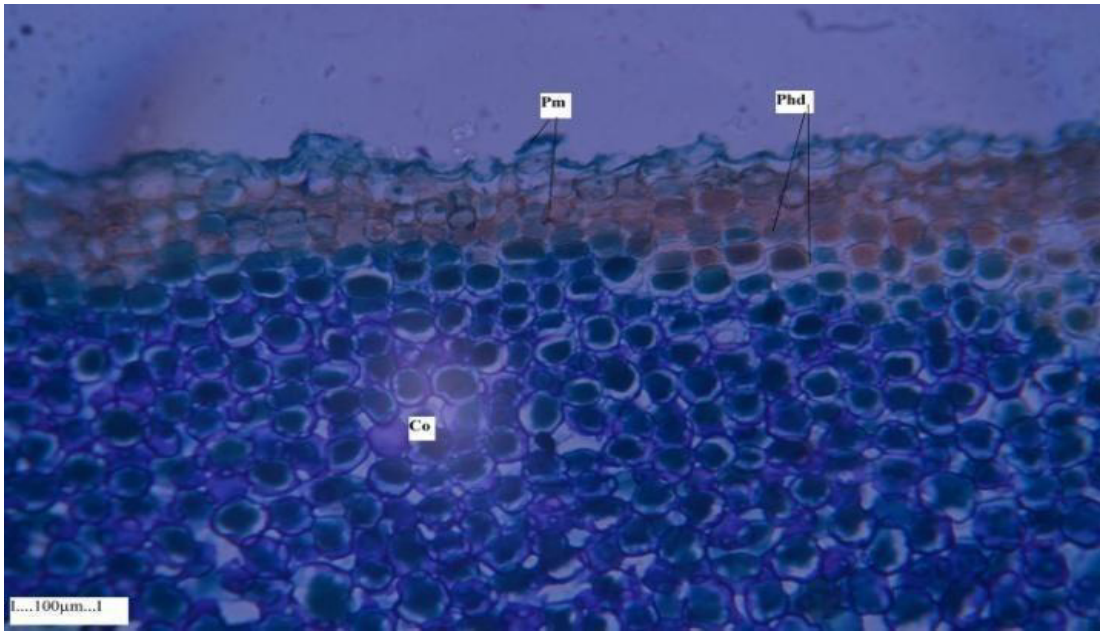

Fig 4.1. T.S of periderm

Co - Cortex, Pm –Phellem, Phd–Phelloderm

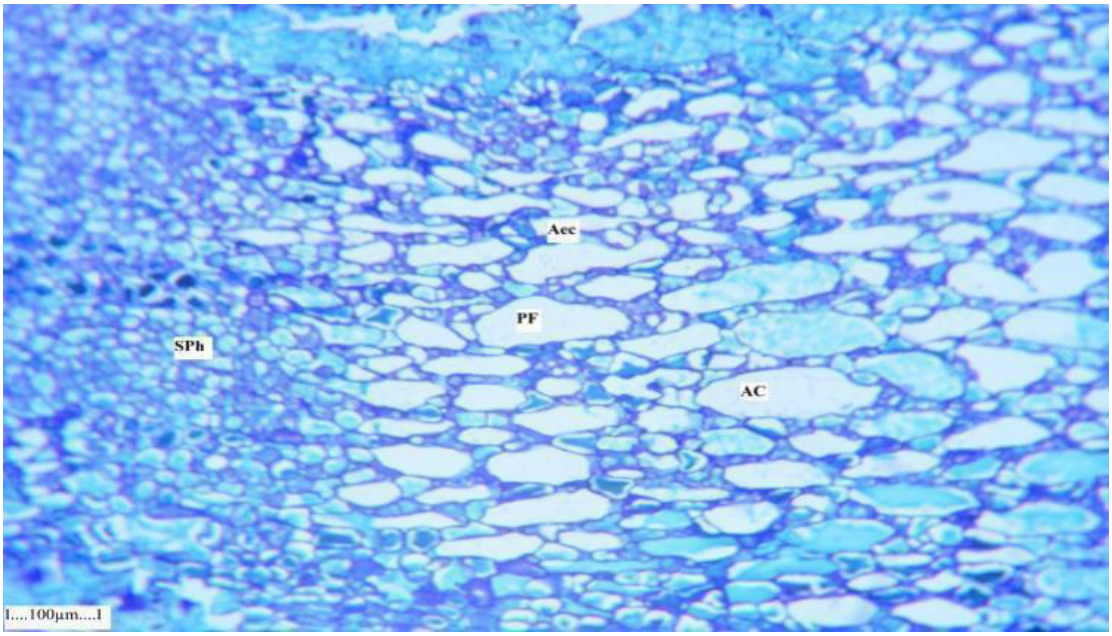

Fig 4.2. T.S of bark showing collapsed zone of phloem

Aec–Radially oblong aerenchyma, SPh –Secondary phloem, PF–Partition filament, AC – Air chamber

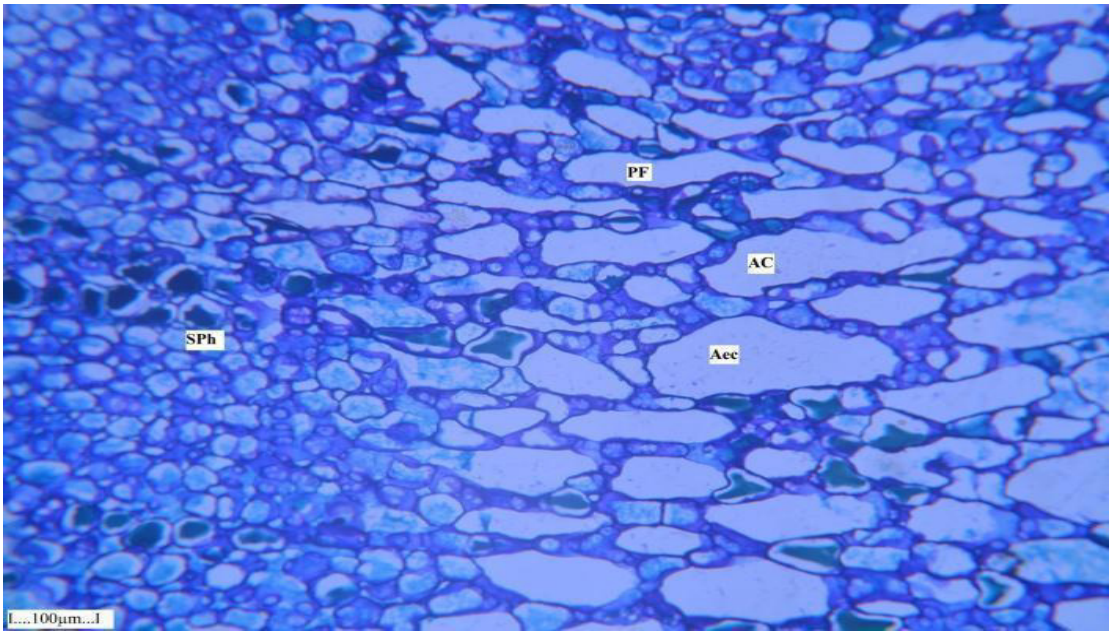

Fig 5.1 T. S of bark showing Aerenchyma and Secondary phloem

Aec–Radially oblong aerenchyma, SPh –Secondary phloem, PF–Partition filament, AC – Air chamber

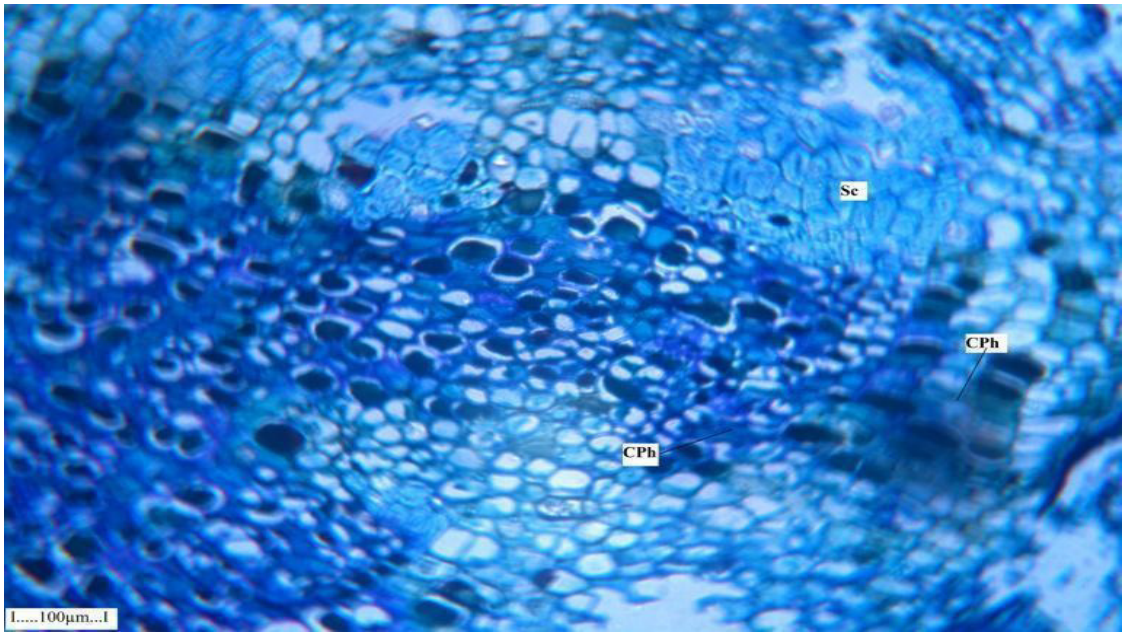

Fig 5.2 T.S of bark showing Aerenchyma and Secondary phloem

Sc-Sclereids, CPh –Collapsed phloem,

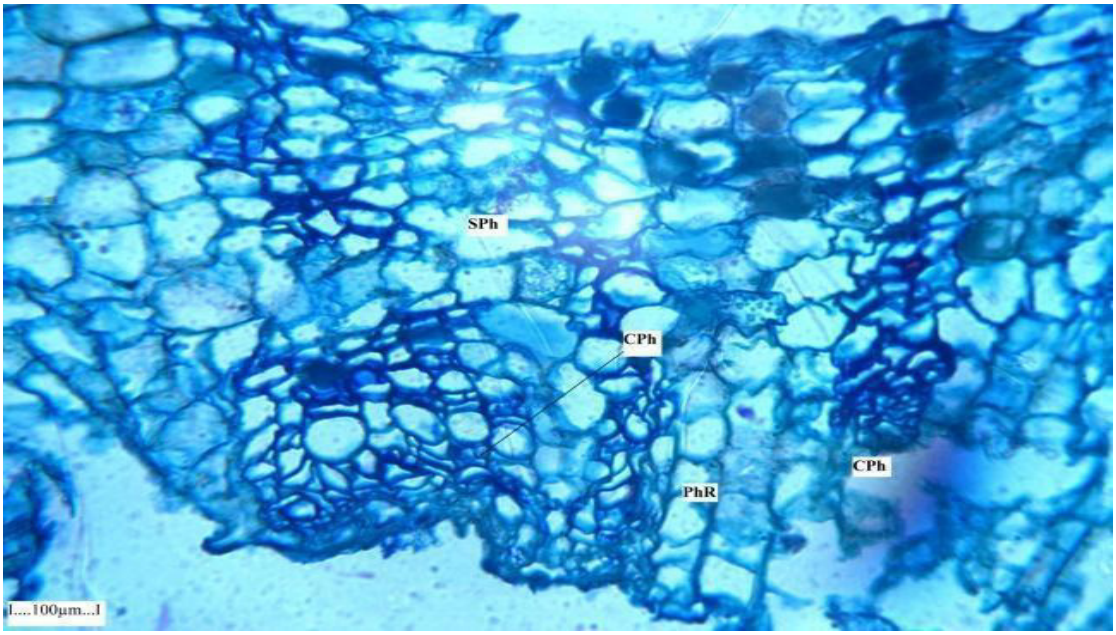

Fig 6.1 T. S of Bark-Collapsed phloem tissues

SPh-Secondary phloem, CPh - Collapsed phloem, PhR –Phloem ray

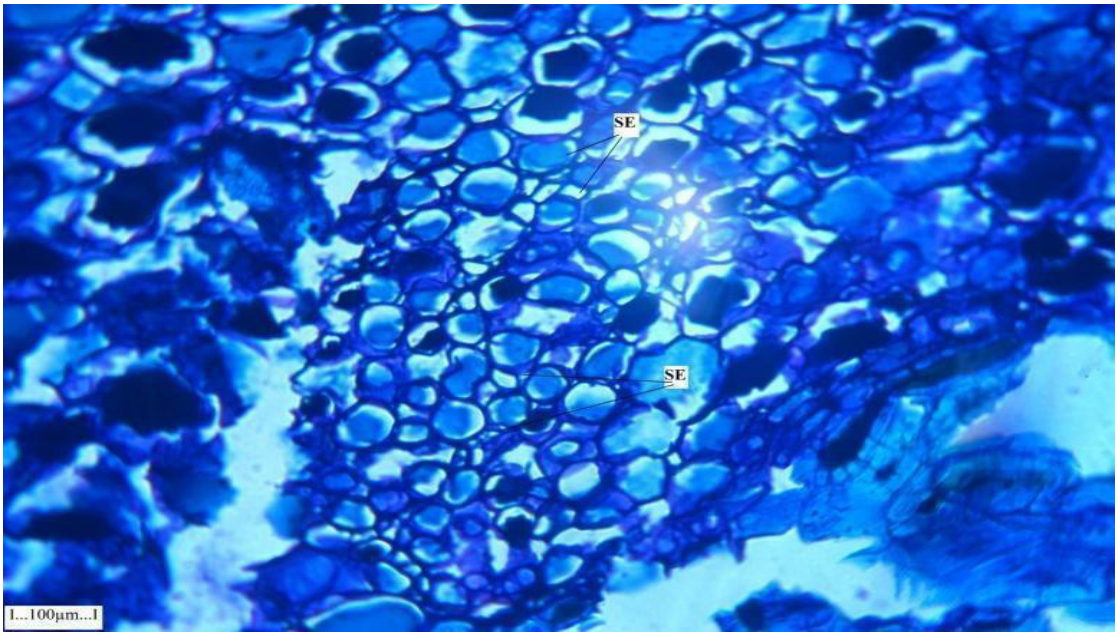

Fig 6.2 T. S of bark-Non collapsed phloem tissues

*SE- **Sieve elements***

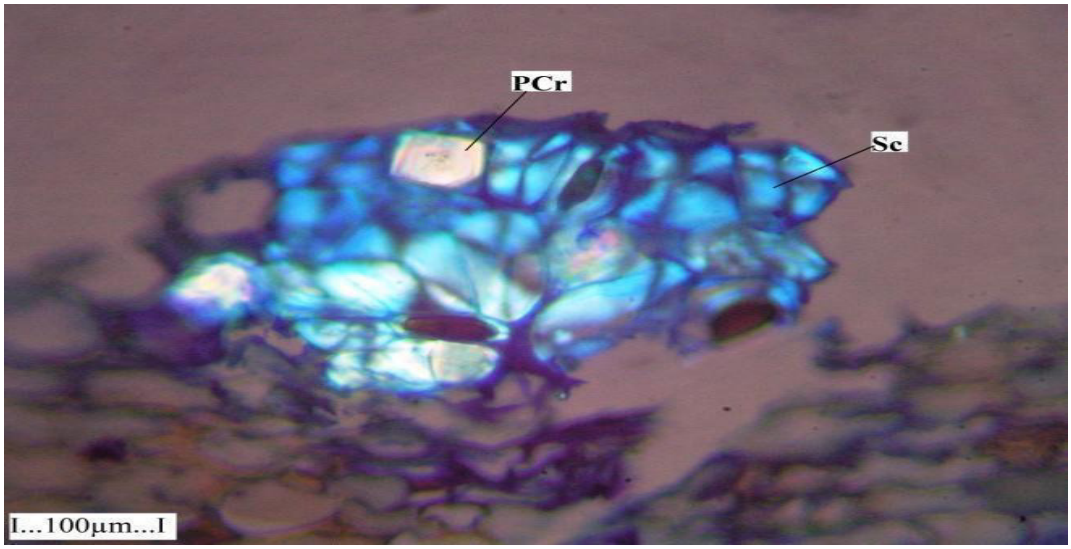

Fig 7.1 Druses type crystals in the cortical parenchyma

PCr- Prismatic crystals, Sc- Sclereids

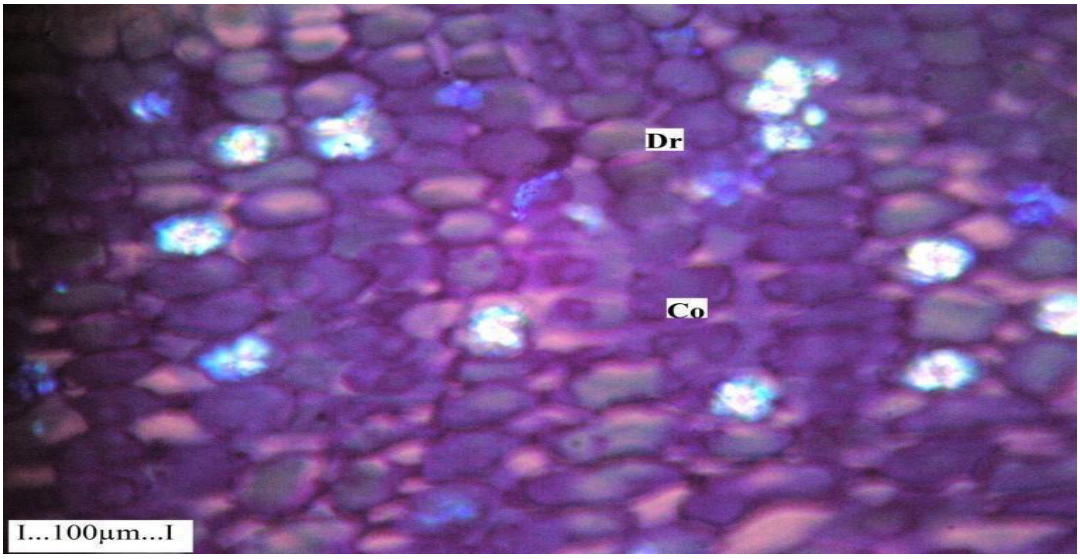

Fig 7.2 Prismatic crystals in the sclereide

Dr- Druces, Co- Cortex

3.8 Powder Characters

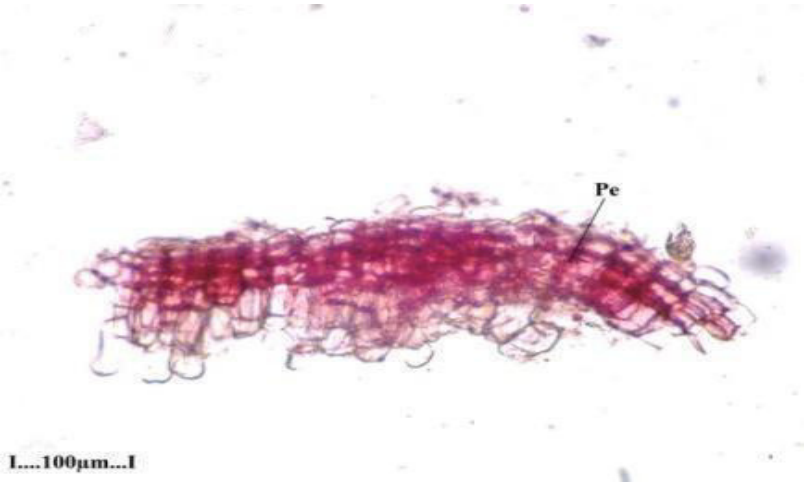

Fig 8.1Fragment of periderm layer

Pe- Periderm

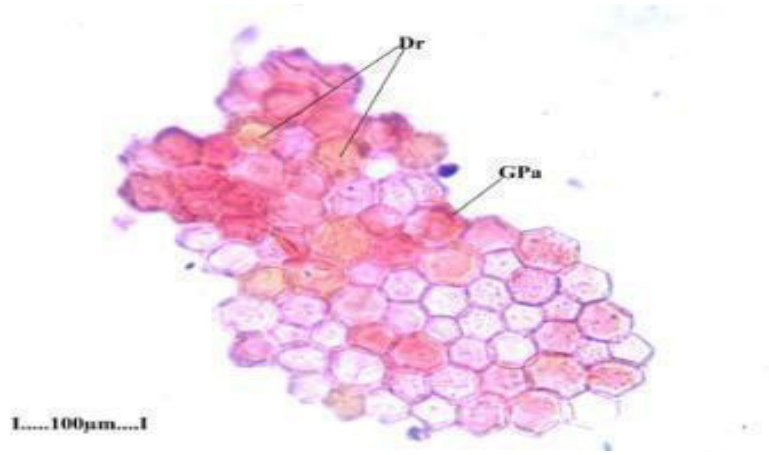

Fig 8.2 Ground paranchyma cell

Dr – Druses, GPa – Ground parenchyma

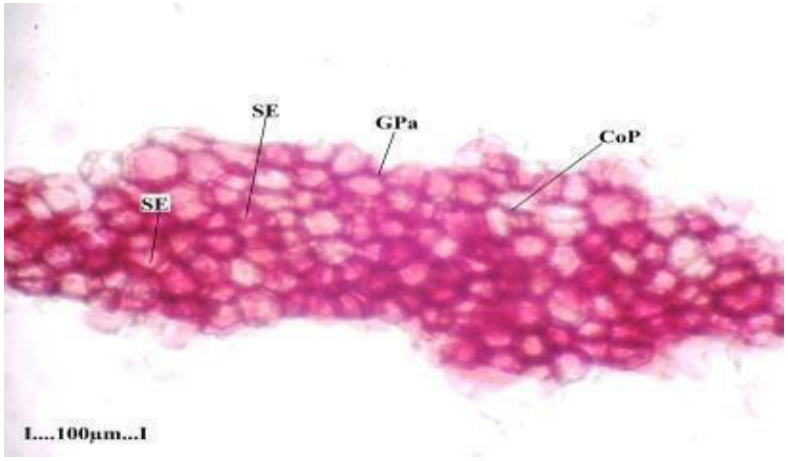

Fig 8.3 Fragment of secondary phloem showing sieve elements

SE – Seive elements, GPa – Ground parenchyma, CoP – Cortical parenchyma

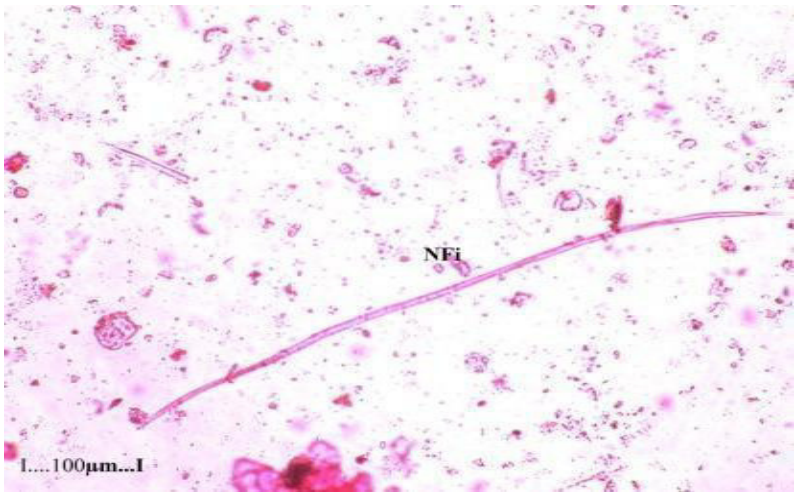

Fig 9.1 Narrow liberiformfibers

NFi - Narrow liberiformfibers

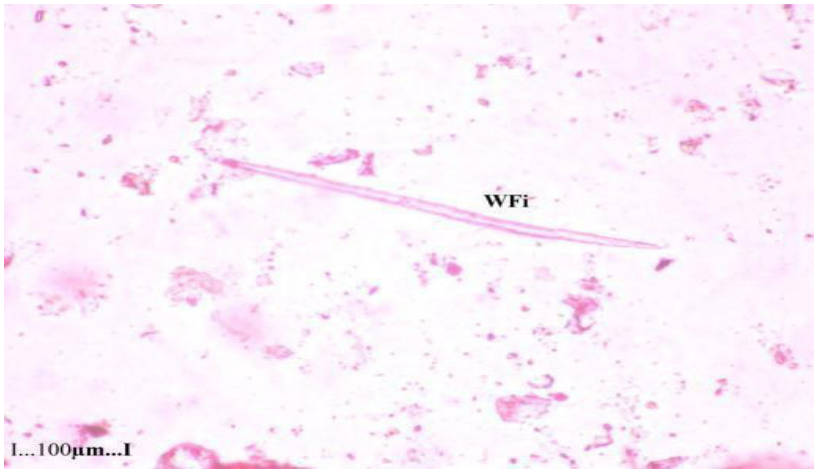

Fig 9.2. Wide libriformfiber

WFi - Wide libriformfiber

4. DISCUSSION

Studying the microscopic characteristics in powdered bark helps the authentication of the bark in powdered conditions. *Kandelia candel* is one of the true mangrove species found in South East Asia. The bark of the plant is traditionally used for tanning leather and dyeing, medicinally the bark of the plant shows anti-oxidant properties, and the bark mixed with dried ginger or long pepper and rose water is considered useful for the treatment of Diabetes.[28] Morphologically bark of the plant shows a greyish to reddish-brown colour. It is curved and sometimes recurved; the inner surface of the bark is slightly yellowish-red these morphological features help in the identification of the bark[29]. Histology of the bark shows a more or less smooth epidermal layer and sometimes which is found broken and periderm is exposed. Periderm consists of 2-5 layers of suberised dead phellem cells which show brown colour on staining. The cortical zone comprises several layers of circular cells having narrow intercellular spaces. The Interior of the bark consists of wide radially oblong aerenchyma tissues, secondary phloem elements, and ground parenchyma was seen on either side of phloem parenchyma cells. Calcium oxalate cells are common in the bark; they are found in the form of druces and prism types. The powder microscopy of the bark shows a small fragment of the periderm, and cortical parenchyma cells some of the cells possess calcium oxalate druces. Long narrow fibers and wider fibers are also found in the powder the dimensions of the fibers were measured and found to be 350.5µm to 911.3µm in length and 14.02µm to 28.04µm breadth the histological characters and powdered microscopic features helps to set a standard for the identification and authentication of the bark and its powder[30]. Physicochemical parameters also help in the authentication of the crude drugs moisture content is very critical in many crude drugs, especially the drugs which are intended for storage for long periods because it promotes oxidation and activation of many enzymes present in plants and subsequent deterioration of the quality of the crude drugs, moisture content also promote microbial growth and causes the spoilage of drugs on long storage so it is preferable to keep the moisture content at a minimum level to preserve the quality of the drug, the physicochemical parameters such as total ash, acid insoluble ash, water-soluble ash and extractives like water-soluble extractive, alcohol, and ether soluble extractives which are fairly constant for a particular crude so it also helps in the authentication of the crude drugs. Total ash represents the mineral content of the drug and acid insoluble ash helps the identification of earthy matters present in drugs which is 1.02%w/w in the bark low value indicates minimal contamination with siliceous matters, and water-soluble ash helps to detect whether the material exhausted by water. Extractive values are used to identify the nature of chemical constituents present in the drug. The bark of *Kandelia candel* shows a higher alcohol soluble extractive value, it indicates that the bark of the plant consists of more polar constituents. The swelling factor helps in the evaluation of crude drugs containing mucilage, foaming index helps in the identification of saponins[31-33]. Botanicals consist of various kinds of phytoconstituents with diversified biological actions that can be useful for the treatment of the various ailments of the humans, these phytochemicals are the secondary metabolites produced by the plants during their life process. Plant metabolites like flavonoids, alkaloids, tannins, saponins, steroids, and terpenoids possess anti-inflammatory effects.[34-38] Tannins, alkaloids glycosides, and flavonoids have been reported to possess anti-diabetic activity.[39,40]Saponins and terpenoids were also effective to reduce the blood sugar level.[41,42]Saponins were also found to be useful for reducing cholesterol levels and they also possess some activities on the central nervous system.[43] Steroids showed to have analgesic and some activities on the central nervous system, Triterpenoids were also found to be effective as an analgesic.[44,45] Preliminary Phytochemical screening of the various bark extracts of the *Kandelia candel* showed the presence of some important phytochemicals like Tannins, flavonoids, steroids, phenols, terpenoids, and glycosides. Traditionally the bark of this plant was found to be useful for reducing the blood sugar level; it may be due to the presence of tannins, flavonoids, and phenolic compounds. Fluorescence is one of the important parameters of pharmacognostic evaluation, plants have diversified types of phytoconstituents, and many of these compounds show fluorescence in different conditions such as daylight and ultraviolet light, but certain compounds didn't show any fluorescence but on treatment with certain chemicals they can be converted to fluorescent derivatives, hence we can rely fluorescent analysis as a method of qualitative evaluation of plant drugs.[46] Fluorescent evaluation of the powdered bark of the *Kandelia candel* shows characteristic colors on treatment with various reagents. HPTLC analysis of the ethanolic extract of the bark of *Kandelia candel* showed the presence of different

phytochemicals in different concentrations. The chromatogram was scanned at all wavelengths representing 7 peaks which indicate the presence of different phytoconstituents, each peak on the chromatogram represents a phytoconstituent. The Rf value calculated (Table 5) was helpful for the identification of different phytochemicals present in the sample by comparing with standards, and the concentration of the different compounds can be determined from the peak area. The preliminary phytochemical screening of the various extracts of the bark showed the presence of flavonoids, tannins, steroids, terpenoids, and phenols. Traditionally the bark of the plant was used for the cure of diabetes so it is evident that various important phytochemicals are present in the bark of *Kandelia candel.* Present HPTLC analysis is limited to the estimate the different phytoconstituents present in the ethanolic extracts of the bark of *Kandelia candel* and it can be determined from the peaks of the chromatogram and peak tables. identification of the unknown compound was not done in the present study[47]. Studying the pharmacognostic features and various physicochemical constants helps to setting the standards for the bark of *kandelia candel* which helps in the identification and authentication of the bark. These parameters are useful for the evaluation and to find out adulteration with similar barks and powdered material of the other barks and also helps to prepare the monograph.

5. CONCLUSION

The World Health Organisation announced the need for the quality control measure of herbal drugs. About 80% of the world population still relies on natural medicine for their primary health care needs. Most of these herbal preparations used in different systems of medicine utilize the large number of crude drugs in their preparation so settings up the pharmacognostic and physicochemical standards for these crude helps in the identification and authentication of genuine drugs and is useful for the detection of foreign matters and admixture of biosimilar materials and these parameters also helps in the preparation of the monograph.

6. AUTHORS CONTRIBUTION STATEMENT

Dr.Malarkodi Velraj and M. Muhammed Habeebulla contributed to the design and implementation of the research, to the analysis of the results and to the writing of the manuscript.

7. CONFLICT OF INTEREST

Conflict of interest declared none.

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